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Anti-microbial resistance to β-lactams and prevalence of colicin genes among phylotypes of *Escherichia coli* isolates from hedgehogs

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Abstract

Several bacteria, such as *Escherichia coli*, *Pseudomonas*, and *Staphylococcus* are considered as indicators of anti-microbial resistance (AMR) in a wide range of hosts and environments, because they may transfer AMR genes to important pathogenic bacteria. Hedgehog is one of the most important wild species living in urban areas. So, this study aimed to determine AMR against β-lactams and prevalence of colicin genes among various phylotypes of *E. coli* isolates from hedgehogs in the Kerman, Iran. Totally, 105 *E. coli* isolates were obtained from 21 hedgehogs (five isolates from each hedgehog). Resistances to the ampicillin (79.10%), cefotaxime (66.70%), and amoxicillin-clavulanate (62.00%) were the most prevalent, and resistance against ceftiofur (39.10%), ceftazidime (39.10%), and ceftriaxone (34.30%) had the lowest prevalence rates. In phenotypic tests, 2.90% of the isolates were extended spectrum β-lactamase producers. The prevalence of β-lactam resistance genes was 26.60% for bla_{TEM}, 3.80% for $bla_{CTX\text{-}M}$, 8.50% for bla_{SHV}, and 1.90% for bla_{CMY}. The frequency of colicin genes, including *E1*, *V*, *E2-E9*, and *Ia.Ib* was 5.71, 4.76, 10.47, and 11.42%, respectively. All *E. coli* isolates were negative for *5.10.K*, *Y.U*, and *A.N.S4* genes. Phylogenetically, B1 (49.50%), A (40.90%), and D (5.70%) were identified among the isolates, and 3.80% remained unknown. Wildlife could be considered as a bio-marker to determine the environmental dissemination of AMR. Also, hedgehog may be an important reservoir of antibiotic-resistant and non-pathogenic *E. coli* strains in urban environments. This study highlights the necessity of *E. coli* surveillance among domestic and wild animals.

Introduction

Recently, various studies have shown the important role of wildlife in the epidemiology of antibiotic-resistant bacterial strains. Anti-microbial resistance (AMR), one of the top global public health threats, occurs when microbes no longer respond to agents, including antibiotics, anti-virals, anti-fungals, and anti-parasitics. The prevalence of resistant bacteria in wildlife is linked with human activity, while it is documented that particular lineages of methicillin-resistant *Staphylococcus aureus* emerged in European hedgehogs in the pre-antibiotic era and subsequently spread to various hosts.[1] A study showed that 36.80% of the wild European hedgehogs carry *Klebsiella pneumoniae*, *Citrobacter freundii*, and *Escherichia coli* strains, being positive for β-lactamase/carbapenemase resistance genes.[2]

Antibiotic resistance and some other characteristics may lead to the survival of *E. coli* in different environmental conditions. One of these features is the ability to produce colicins being mainly encoded by plasmids; colicins are produced by *Enterobacteria* and lead to rapid elimination of susceptible cells, giving them a competitive advantage in the environment.[3] Various environmental conditions create some natural selective pressure on *E. coli* populations, leading to divergence and diverse genetic sub-structures within the species. So, *E. coli* can be divided into different phylogenetic groups, such as A, B1, B2, C, D, E, F, and clade I.[4] The extended Clermont *et al.* classification is an updated system using a combination

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of phylogenetic markers, including *chuA*, *yjaA*, *TspE4.C2*, and *arpA* to amplify target-specific fragments using polymerase chain reaction (PCR).[4]

This study aimed to investigate the prevalence of β-lactam resistant *E. coli* strains in hedgehogs from the Kerman region of Iran, exploring their potential as a reservoir for humans. Also, this study tried to understand the nature of isolated *E. coli* strains by screening of phylogenetic and colicinogenic sequences. This information may be crucial for implementing strategies to prevent further development and spread of AMR between animals and humans.

Materials and Methods

Sampling and *E. coli* isolation. In this study, fresh fecal samples were collected from 21 hedgehogs over 2 months in the spring (April and May 2022). Each hedgehog was kept overnight, and its feces were collected in sterile containers. The samples were stored at 4.00 ˚C and transported to the Microbiology Laboratory at the Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran, within 24 hr using ice packs. Finally, each hedgehog was marked with dye to prevent repeated sampling and released. Fecal samples were cultured on MacConkey agar (Merck, Darmstadt, Germany) and aerobically incubated at 37.00 ˚C for 24 to 48 hr Suspected colonies of *E. coli* (*i.e.*, those appeared round, smooth, and pink) were selected for biochemical confirmation using indole, methyl red, Voges-Proskauer, and citrate indole, methyl red, Voges-Proskauer, and citrate tests. Finally, confirmed *E. coli* isolates were used for subsequent analyses.[5]

Phenotypic assessment of resistance to β-lactams. In this study, the AMR was identified using the Kirby Bauer disk diffusion method;[6] the antibiotics (Padtan Teb co., Tehran, Iran) were cefotaxime (CTX; 30.00 µg), ceftriaxone (CRO; 30.00 µg), cefixime (CFM; 30.00 µg), ceftazidime (CAZ; 30.00 µg), ceftiofur (CEF; 30.00 µg), ampicillin (AM; 30.00 µg), and amoxicillin-clavulanate (AMC; 30.00 µg). As a test control, *E. coli* ATCC 25922 was utilized, and the findings were evaluated according to the Clinical and Laboratory Standards Institute.[7] Also, the *E. coli* strains with a ≥ 5.00-mm increase in zone diameter for CTX-clavulanate *versus* the zone diameter of CTX or a ≥ 5.00-mm increase in zone diameter for CAZ -clavulanate *versus* the zone diameter of CAZ were considered as extended spectrum β-lactamase (ESBL)-producing *E. coli* strains.[8] The diameter of the zone of inhibitions was measured and recorded in millimeters, and the organism was labeled as sensitive, resistant, or intermediate based on following guidelines:[7] CTX (resistant ≤ 22.00 mm and 26.00 mm ≤ sensitive), CRO (resistant ≤ 19.00 mm and 23.00 mm ≤ sensitive), CFM (resistant ≤ 14.00 mm and 15.00 mm ≤ sensitive), CAZ (resistant ≤ 17.00 mm and 21.00 mm ≤ sensitive), CEF (resistant ≤ 16.00 mm and 27.00 mm ≤ sensitive), AM (resistant ≤ 13.00 mm and 17.00 mm ≤ sensitive), and AMC (resistant ≤ 13.00 mm and 18.00 mm ≤ sensitive).

Polymerase chain reaction for β-lactamase genes. Single and confirmed *E. coli* colonies were suspended in 400 µL of sterile distilled water and heated to 98.00 - 100 ˚C for 10 - 15 min in a heating block (Eppendorf, Hamburg, Germany). The suspension was then cooled on ice for 10 min and subsequently centrifuged at 13,000 *g* for 1 min. The resulting supernatant, containing DNA, was used as a template in the PCR.[9] For evaluating the quality and quantity of DNA extraction, absorbance of the DNA templates was measured at optical density (OD) 260 and OD 280 using a NanoDrop (BioTek, Winooski, USA). The OD 260/280 ratio was used as an indicator of DNA purity. Extracted DNA samples contained at least 1.00 ng *per* µL and had an OD 260/280 ratio between 1.80 and 2.00 were selected for subsequent steps. Four AMR genes, including *bla*$_{TEM}$ (964 bp), *bla*$_{SHV}$ (795 bp), *bla*$_{CMY}$ (1,146 bp), and *bla*$_{CTX\text{-}M}$ (585 bp), were screened by PCR.[10] Uniplex PCR was carried out in 25.00 µL reactions, containing 12.50 µL 2.00 X Taq PCR Master Mix (Pars Tous, Mashhad, Iran), 0.40 µM of each forward and reverse primer (Table 1),[10] 8.00 µL of sterile water, and 2.50 µL of extracted bacterial DNA. The PCR steps were initial denaturation (95.00 ˚C for 5 min), and 35 thermal cycles each including denaturation (95.00 ˚C for 30 sec), annealing (55.00 ˚C, 30 sec for *bla*$_{TEM}$ and *bla*$_{SHV}$ and 60.00 ˚C, 30 sec for *bla*$_{CTX\text{-}M}$ and *bla*$_{CMY}$), and elongation (72.00 ˚C for 1 min). Finally, an elongation step was attached at 72.00 ˚C for 5 min. Positive controls were *Klebsiella* ATCC 700603 (for *bla*$_{CTX\text{-}M}$), *E. coli* ATCC 35218 (for *bla*$_{TEM}$ and *bla*$_{SHV}$), and a clinical positive sample from our previous projects (for *bla*$_{CMY}$), and distilled water was used as a negative control.

Polymerase chain reaction for colicin genes. Colicin genes (*Y.U* [243 bp], *E1* [389 bp], *V* [400 bp], *5.10.K* [803 bp], *E2-E9* [219 bp], *Ia.Ib* [385 bp], and *A.N.S4* [225 bp]) were screened in the strains.[11] For the PCR steps, the reactions were uniplex and arranged in 25.00 µL volume for each gene, including 3.00 µL DNA extract, 2.50 µM of each primer (Table 1), 2.50 µL 2.00 X Taq PCR Master Mix (Pars Tous), and distilled water up to the volume of the reaction. The PCR steps were initial denaturation (94.00 ˚C for 5 min), and 35 thermal cycles, including denaturation (94.00 ˚C for 2 min), annealing (57.00 ˚C for 30 sec [for *A.N.S4*], 60.00 ˚C for 30 sec [for *5.10.K* and *E2-E9*], and 60.00 ˚C for 30 sec [for *Y.U*, *E1*, *V*, and *Ia.Ib*]), and elongation (72.00 ˚C for 1 min). Finally, an additional elongation step was included at 72.00 ˚C for 5 min. Clinical positive samples from our previous projects were used as positive controls and distilled water was used as a negative control.

Table 1. List of primers used in this study.

Gene target	Primer sequences (5′-3′)	Product size (bp)	Reference
bla_{TEM}	F: GCGGAACCCCTATTTG R: ACCAATGCTTAATCAGTGAG	964	9
bla_{SHV}	F: TTATCTCCCTGTTAGCCACC R: GATTTGCTGATTTCGCTCGG	795	9
$bla_{CTX\text{-}M}$	F: CGATGTGCAGTACCAGTAA R: TTAGTGACCAGAATCAGCGG	585	9
bla_{CMY}	F: ATGATGAAAAAATCGTTATGCT R: TTATTGCAGCTTTTCAAGAATGCG	1,146	10
A.N.S4	F: CGTAGCTATAATGAAGCAATGGCTTCA R: ACCTCCAACAGGAGAGGTCCCCAGTT	225	11
V	F: CACGCCCTGAAGCACCACCA R: CCGTTTTCCAAGCGGACCCC	400	11
Ia.Ib	F: GCACAACAGGCCCGTCTGCTC R: CACCTTCCACATCCTCTGTCACC	385	11
E2-E9	F: CGACAGGCTAAAGCTGTTCAGGT R: TGCAGCAGCATCAAATGCAGCCT	219	11
Y.U	F: GTGAACGGACAGAAACCCGCC R: CAATCTGTCTGACAGCCTCTCCC	243	11
5.10.K	F: AAAGCTGAACTGGCGAAGGC R: CAACTCATCATCCCCTATGTAAGAAG	803	11
E1	F: ACGGGAGTGGCTCTGGCGG R: CTCTTTACGTCGTTGTTCTGCTTCCTG	389	11
chuA	F: ATGGTACCGGACGAACCAAC R: TGCCGCCAGTACCAAAGACA	288	12
yajA	F: CAAACGTGAAGTGTCAGGAG R: AATGCGTTCCTCAACCTGTG	211	12
TSPE4C2	F: CACTATTCGTAAGGTCATCC R: AGTTTATCGCTGCGGGTCGC	152	12
arpA	F: AACGCTATTCGCCAGCTTGC R: AACGCTATTCGCCAGCTTGC	400	12

Polymerase chain reaction for phylogenetic classification. In this study, the *E. coli* strains were phylotyped by Clermont *et al.* scheme using a quadriplex PCR (Table 1).[12] The sequences *arpA* (400 bp), *chuA* (288 bp), *yjaA* (211 bp), and *TspE4.C2* (152 bp) were targeted to determine phylogenetic groups, including A, B1, B2, C, D, E, F, and cryptic clade I. The EcoR62 and EcoR20 strains were used as positive controls in PCR examinations. Samples were subjected to a 35 PCR cycles, including 10 sec denaturation at 94.00 ˚C, 25 sec annealing at 59.00 ˚C, and five sec elongations at 72.00 ˚C. The PCR products were electrophoresed on 1.50% agarose gel for 45 min at 90 V. The electrophoresed gel was analyzed by Gel Documentation Imaging System (version ST4; Vilber Lourmat Co., Marne-la-Vallée, France).

Results

***Escherichia coli* isolation and phenotypic AMR.** In total, 105 *E. coli* isolates were obtained from 21 hedgehogs; all statistical frequencies were measured based on the 105 isolates. The results showed that resistances to the AM (79.10%; 95.00% confidence interval (CI): 70.00 - 86.30%), CTX (66.70%; 95.00% CI: 56.80 - 75.50%), and AMC (62.00%; 95.00% CI: 51.90 - 71.20%), were the most prevalent. Resistance against three antibiotics, including CEF (39.10%; 95.00% CI: 29.60 - 49.00%), CAZ (39.10%; 95.00% CI: 29.60 - 49.00%), and CRO (34.30%; 95.00% CI: 25.30 - 44.10%) had the lowest prevalence rates (Fig. 1A). In this study, 96/105 isolates (91.60%; 95.00% CI: 84.30 - 96.00%) showed phenotypic resistance to at least one of the studied antibiotics. There were 32 phenotypic resistance patterns; some patterns showed co-resistance against up to six antibiotics (Table 2). The most common resistance pattern was related to CAZ, AM, CEF, CTX, CRO, AMC, and AM, CTX, AMC (Table 2). In phenotypic tests, three isolates (2.90%; 95.00% CI: 0.50 - 8.10%) were observed to be ESBL producers (Fig. 1D and Fig. 4).

Prevalence of β-lactamase genes. Among 105 isolates, 28 isolates (26.60%; 95.00% CI: 17.70 - 38.50%) were positive for the *bla_{TEM}* gene, four isolates (3.80%; 95.00% CI: 1.00 - 9.40%) were positive for the *$bla_{CTX\text{-}M}$* gene, nine isolates (8.50%; 95.00% CI: 3.91 - 16.20%) were positive for the *bla_{SHV}* gene, and two isolates (1.90%; 95.00% CI: 0.20 - 6.80%) were positive for the *bla_{CMY}* gene (Fig. 1B and Fig. 2). One of the *E. coli* isolates was positive for three resistance genes, including *bla_{TEM}*, *bla_{SHV}*, and *bla_{CMY}*, simultaneously (Table 3).

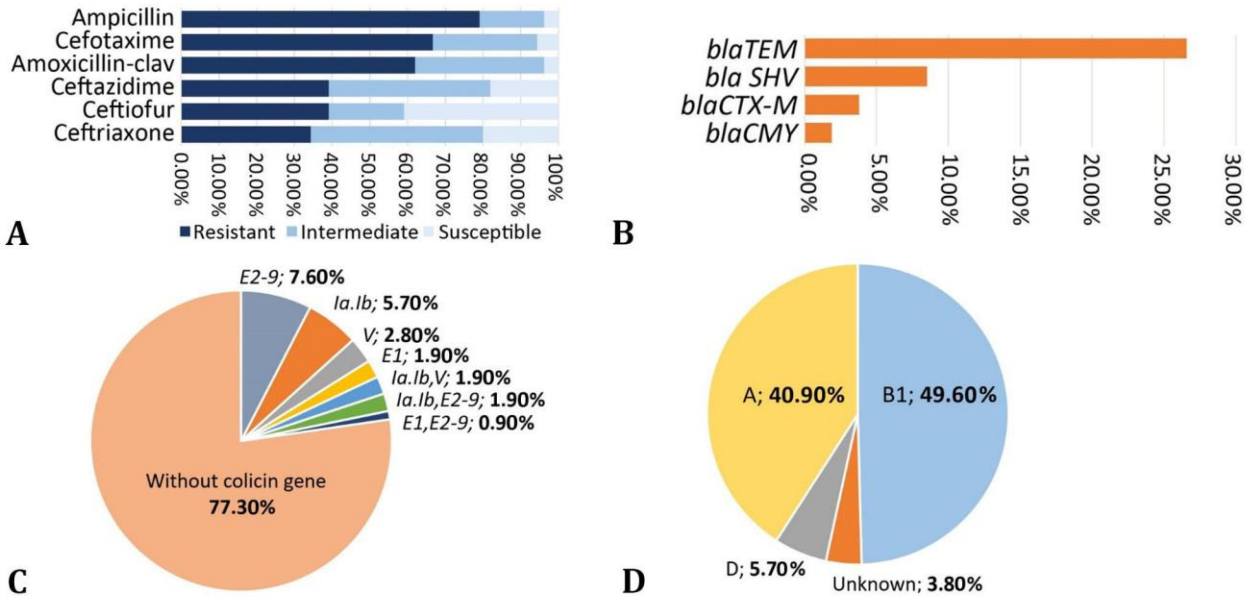

Fig. 1. Prevalence of **A)** Phenotypic, and **B)** Genotypic resistances, **C)** Colicin genes, and **D)** Phylogenetic groups in *Escherichia coli* isolates from hedgehogs.

Table 2. Prevalence of phenotypic resistance patterns in *Escherichia coli* isolates from hedgehogs.

Phenotypic anti-microbial resistance patterns	Combination of antibiotics	No. isolates (%)	95.00% CI
CAZ, AM, CEF, CTX, CRO, AMC	6	13 (12.40)	6.70 - 20.20
AM, CEF, CTX, CRO, AMC	5	6 (5.70)	2.10 - 12.00
CAZ, AM, CEF, CTX, AMC	5	5 (4.80)	1.50 - 10.70
CAZ, AM, CEF, CTX, CRO	5	2 (1.90)	0.20 - 6.70
CAZ, AM, CTX, CRO, AMC	5	2 (1.90)	0.20 - 6.70
CAZ, AM, CTX, AMC	4	7 (6.70)	2.70 - 13.20
AM, CTX, CRO, AMC	4	4 (3.80)	1.00 - 9.40
CAZ, AM, CEF, AMC	4	2 (1.90)	0.20 - 6.70
AM, CEF, CTX, AMC	4	2 (1.90)	0.20 - 6.70
AM, CEF, CRO, AMC	4	2 (1.90)	0.20 - 6.70
CEF, CTX, CRO, AMC	4	1 (0.95)	0.00 - 5.10
CAZ, CEF, CTX, CRO	4	1 (0.95)	0.00 - 5.10
CAZ, AM, CTX, CRO	4	1 (0.95)	0.00 - 5.10
AM, CEF, CTX, CRO	4	1 (0.95)	0.00 - 5.10
AM, CTX, AMC	3	10 (9.55)	4.60 - 16.80
CAZ, AM, CTX	3	3 (2.80)	0.50 - 8.10
AM, CEF, CTX	3	2 (1.90)	0.20 - 6.70
AM, CRO, AMC	3	2 (1.90)	0.20 - 6.70
AM, CEF, AMC	3	1 (0.95)	0.00 - 5.10
CAZ, AM, AMC	3	1 (0.95)	0.00 - 5.10
CEF, CTX, AMC	3	1 (0.95)	0.00 - 5.10
AM, CTX	2	5 (4.80)	1.50 - 10.70
CAZ, AM	2	2 (1.90)	0.20 - 6.70
CAZ, CTX	2	1 (0.95)	0.00 - 5.10
AM, CEF	2	1 (0.95)	0.00 - 5.10
CTX, CRO	2	1 (0.95)	0.00 - 5.10
AM, AMC	2	1 (0.95)	0.00 - 5.10
CAZ, AMC	2	1 (0.95)	0.00 - 5.10
AM	1	8 (7.60)	3.30 - 14.40
AMC	1	4 (3.80)	1.00 - 9.40
CTX	1	2 (1.90)	0.20 - 6.70
CEF	1	1 (0.95)	0.00 - 5.10
Without phenotypic resistance	0	9 (8.60)	3.90 - 15.60
Total	-	105 (100)	-

CAZ: Ceftazidime; AM: Ampicillin; CTX: Cefotaxime; CRO: Ceftriaxone; CEF: Ceftiofur; AMC: Amoxicillin-clavulanate, CI: Confidence interval.

Colicins. Six isolates out of 105 isolates (5.71%; 95.00% CI: 2.10 - 12.00%) had *E1* gene, five (4.76%; 95.00% CI: 1.50 - 10.70%) had *V* gene, 11 (10.47%; 95.00% CI: 5.30 - 17.90%) had *E2-E9* gene, and 12 (11.42%; 95.00% CI: 6.00 - 19.10%) had *Ia.Ib* gene (Fig. 1C and Fig. 3). All 105 isolates were negative for *5.10.K*, *Y.U*, and *A.N.S4* genes.

Prevalence of phylogenetic groups. Overall, out of 105 *E. coli* isolates, phylogenetic groups A, B1, and D, and U strains were identified. From high to low prevalence, B1 (52 isolates; 49.50%; 95.00% CI: 39.60 - 59.40%), A (43 isolates; 40.90%; 95.00% CI: 31.40 - 50.90%), and D (six isolates; 5.70%; 95.00% CI: 2.10 - 12.00%) were recorded, and four isolates (3.80%; 95.00% CI: 1.00 - 9.40%) had U phylogenetic background for PCR based phylogroup.

Discussion

Wildlife is a good indicator of environmental health due to its close interaction with human populations and domestic animals. Hedgehogs can be found in rural and urban areas and are occasionally kept as pets. So, this animal could shed various microorganisms to environment. In this study, *E. coli* was detected from feces of all the hedgehogs. Garcias *et al.* have reported various microorganisms from hedgehogs in Spain which *E. coli* was isolated from 38.90% of the animals.[2] The *E. coli* prevalence was 71.00% in a study in The Netherlands and 34.50% in Northwest of Iran.[13,14] This variation of abundance in different studies depends on several factors, such as animals living in the study environment, the season of sampling, and method of microbial identification. Common living environments of hedgehogs with other animals increase the possibility of transmission of various pathogens among different species of wildlife and even domestic animals. So free-living vertebrates in urban and rural ecosystems can act as reservoirs for zoonotic pathogens between humans and animals through direct and indirect contacts. Several outbreaks of infectious agents in humans have been related to hedgehogs, the

Table 3. Prevalence of genotypic resistance patterns in *Escherichia coli* isolates from hedgehogs.

Genotypic anti-microbial resistance patterns	Combination of resistance genes	No. isolates (%)	95.00% CI
bla$_{TEM}$	1	25 (23.80)	15.40 - 35.10
bla$_{SHV}$	1	7 (6.70)	2.60 - 13.70
bla$_{CTX\text{-}M}$	1	3 (2.80)	0.50 - 8.30
bla$_{TEM}$, ***bla***$_{SHV}$	2	1 (0.90)	0.02 - 5.30
bla$_{TEM}$, ***bla***$_{CTX\text{-}M}$	2	1 (0.90)	0.02 - 5.30
bla$_{TEM}$, ***bla***$_{SHV}$, ***bla***$_{CMY}$	3	1 (0.90)	0.02 - 5.30
bla$_{CMY}$	1	1 (0.90)	0.02 - 5.30
Without resistance genes	0	61 (63.10)	44.40 - 74.60
Total	-	105 (100)	-

CI: Confidence interval.

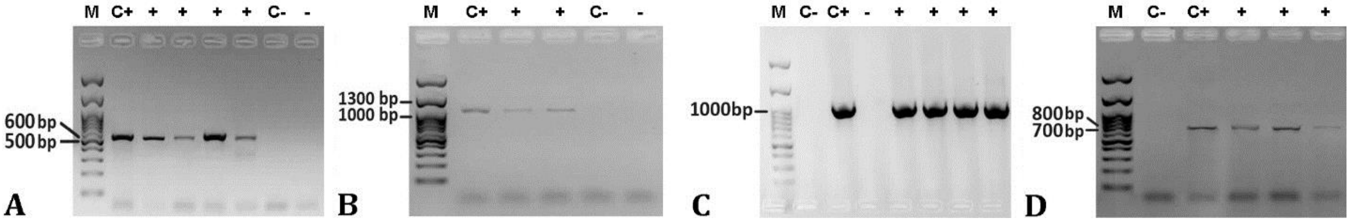

Fig. 2. Gel electrophoresis image of the amplicons obtained from PCR for **A)** *bla*$_{CTX\text{-}M}$ (585 bp), **B)** *bla*$_{CMY}$ (1,146 bp), **C)** *bla*$_{TEM}$ (964 bp), and **D)** *bla*$_{SHV}$ (795 bp) genes. Lanes M: 100 bp marker; Lanes C+: Positive control (*bla*$_{CTX\text{-}M}$ [**A**], *bla*$_{CMY}$ [**B**], *bla*$_{TEM}$ [**C**], and *bla*$_{SHV}$ [**D**]); Lanes C-: Negative control; Lanes +: Positive sample; Lanes -: Negative sample.

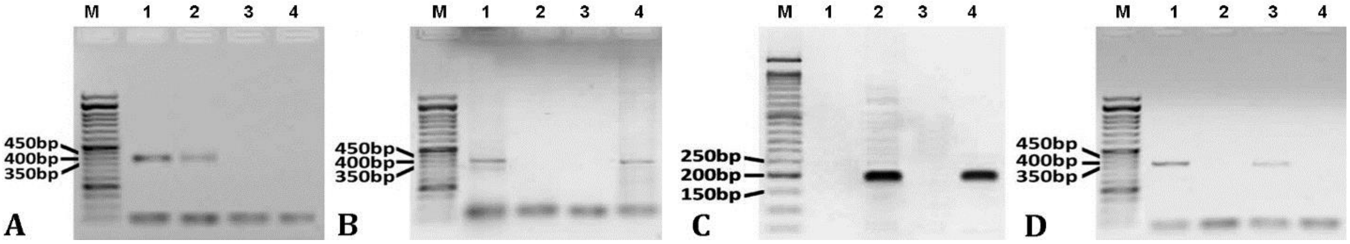

Fig. 3. Gel electrophoresis image of the amplicons obtained from PCR for *E1*, *V*, *E2-E9*, and *Ia.Ib* genes and various phylogroups. **A)** *V* (400 bp): Lane M: 50 bp marker; Lane 1: Positive control; Line 2: Positive sample; Lane 3: Negative sample; Lane 4: Negative control; **B)** *E1* (389 bp): Lane M: 50 bp marker; Lane 1: Positive control; Line 2: Negative control; Lane 3: Negative sample; Lane 4: Positive sample; **C)** *E2-E9* (219 bp): Lane M: 50 bp marker; Lane 1: Negative control; Line 2: Positive control; Lane 3: Negative sample; Lane 4: Negative control; **D)** *Ia.Ib* (385 bp): Lane M: 50 bp marker; Lane 1: Positive control; Line 2: Negative control; Lane 3: Positive sample; Lane 4: Positive control;

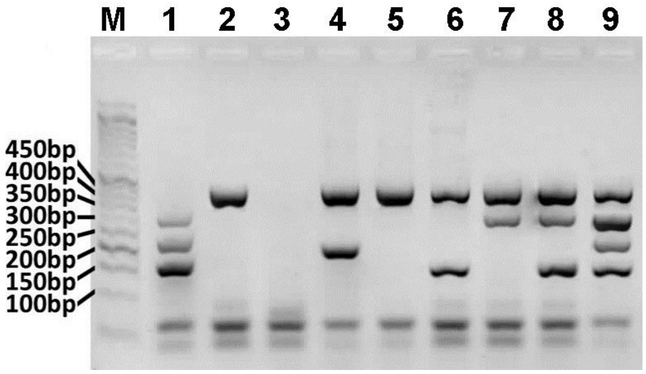

Fig. 4. Gel electrophoresis image of the amplicons obtained from polymerase chain reaction for *E1*, *V*, *E2-E9*, and *Ia.Ib* genes and various phylogroups. Lane M: Marker; Lane 1: Positive control (EcoR62) for *chuA* (288 bp), *yjaA* (211 bp), and *TspE4.C2* (152 bp); Lane 2: Positive control (EcoR20) for *arpA* (400 bp); Lane 3: Negative control; Lane 4: Phylogroup A or C; Lane 5: Phylogroup A; Lane 6: Phylogroup B1; Lanes 7 and 8: Phylogenetic group D or E; Lane 9: Unidentified phylogroup.

most important of which is salmonellosis.[15,16] Pista *et al.* have recovered enteropathogenic *E. coli* pathotype from 5.50% of the 18 hedgehog fecal samples in Portugal.[17] Although the risk of pathogens entering the food chain by wildlife is low, it should not be overlooked.

Recently, resistance against β-lactams has been widely reported from humans, domestic animals, the environment, and wildlife; ESBL-producing *E. coli* strains have also been reported from healthy hosts.[18] Wildlife may acquire anti-microbial agents and residues, resistant bacteria, and resistance genes through human-impacted habitats. Among the β-lactams, third and fourth generation cephalosporins are important antibiotics for the treatment of infectious diseases in humans and animals.[19,20] In the present study, phenotypic and genotypic resistances against β-lactam antibiotics were screened by the various methods. Order of prevalence rates for phenotypic resistance was AM > CTX > AMC > CEF = CAZ > CRO. Zare and Ghorbani-Choboghlo have reported a comparable order of resistance prevalence rate, including AM > AMC > CRO > CFM from northwest of Iran.[14] In a study on AMR of *E. coli* isolated from the rectal swab of African pygmy hedgehog (*Atelerix albiventris*) and sunda porcupine (*Hystrix javanica*) in Indonesia, all isolates were resistant to erythromycin and penicillin G.[21] In Chile, *Salmonella enterica* was detected from 2.50% of ground hedgehogs *(Atelerix albiventris)* which had AMR against AM and CEF.[22] More than 50.00% of *E. coli* isolates from wild European hedgehogs (*Erinaceus europaeus*) living in populated areas of Spain were non-susceptible to AM, AMC, CEF, and CRO.[2] In our study, frequency of ESBL-producing *E. coli* was low (2.80%), being contrary to the results reported from European hedgehogs (16.00%) in suburban and urban areas of The Netherlands.[13]

Due to the widespread use of anti-microbials in agriculture, veterinary medicine, and human medicine, antibiotic residues enter the environment through wastewater and sewage. These residues are received by wildlife in various ways, including contaminated water and soil. Selective pressure associated with antibiotic residues causes the emergence of anti-microbial-resistant bacteria in wildlife microbiota. However, several host-related factors may play a role in the occurrence of resistant strains, including species, age, sex, and diet.[18] In this study, bla_{TEM} and $bla_{CTX\text{-}M}$ genes were the most frequent genes, being comparable with the previous study on Algerian and European hedgehogs (n=50) in Catalonia reporting low to moderate prevalence of bla_{CMY} (*E. coli* [8.00%] and *K. pneumoniae* [10.00%]), $bla_{\text{CTX-M}}$ (*K. pneumoniae* [4.00%]), bla_{SHV} (*E. coli* [4.00%] and *K. pneumoniae* [6.00%]), and bla_{TEM} (*K. pneumoniae* [4.00%]).[23] Also, Garcias *et al.* found lower frequencies in European hedgehogs (n = 114) of Spain, including 7.00, 7.00, 0.80%, and 2.60% for *E. coli* isolates positive for bla_{CMY}, $bla_{CTX\text{-}M}$, bla_{TEM}, and bla_{SHV}, respectively.[2] Hedgehogs are exposed to the bacteria carrying AMR genes and/or anti-microbial residues due to their presence in human areas and livestock in the suburbs.

One of the factors considered in this study was the presence of colicin genes in *E. coli* isolates. Today, colicin and microcin have received a lot of attention, because they can potentially be used as an alternative to antibiotics and colicin/microcin-producing bacteria as a probiotic. However, they may act as a virulence factor.[24] In the present study, 22.70% of the *E. coli* isolates had at least one of the *E1*, *V*, *E2-E*9, and *Ia.Ib* gene groups, and *K.5.10*, *Y.U*, and *A.N.S4* were not found. There aren't any reports about presence of colicinogenic *E. coli* strains in hedgehogs. In a study, 23.60% of *E. coli* strains obtained from farm animals, including chicken, turkey, quail, pig, rabbit, and cow were colicinogenic, including *E1* (52.90%), *Ia* (29.40%), *E9* (23.50%), and *Ib* (23.50%), while the prevalence of the *V*, *5*, *10*, *K*, *E3*, *N*, *U*, and *Y* genes was < 15.00% (30). A study on colicinogenic *E. coli* in fecal samples of human, cow, and sheep in Iran showed that 70.40% of *E. coli* had the *E6* gene followed by *Ib* (66.40%), *E4* (53.60%), and *E7* (49.90%) genes; also, the prevalence of *Ia* (27.90%), *S4* (12.00%), *E3* (13.20%), and *E9* (2.90%) in human samples was more than cows and sheep, whereas *10* (5.80%) and *E5* (4.40%) were produced only by bovine *E. coli* strains.[25] Colicinogenic *E. coli* prevents the colonization of pathogens in the human or animal intestines. Some studies have reported various levels of anti-microbial effects in colicinogenic *E. coli* against avian pathogenic, Shiga toxin-producing, entero-toxigenic, entero-invasive, entero-hemorrhagic, entero-aggregative, and entero-pathogenic, O157:H7, and O26:H11 *E. coli* strains, as well as *Pseudomonas aeruginosa, S. enterica*, and *K. pneumoniae*.[25-27]

Our *E. coli* isolates were mostly classified into three phylogenetic groups, including A, B1, and D, which B1 and

A had the highest prevalence, indicating the dominance of the strains with a non-pathogenic nature. The commensal and non-pathogenic *E. coli* strains are considered to be indicators for AMR in a wide range of Gram-negative bacteria, because these commensal and antibiotic-resistant strains may transfer AMR genes to important pathogenic bacteria in humans and animals by different methods of horizontal gene transfer.[28] Our findings are in agreement with a study conducted by Alonso *et al.*, reported B1 and A phylogroups from *E. europaeus*.[29] Kwaśna *et al.* detected *E. coli* isolates from black grouse (*Lyrurus tetrix*) in the Karkonosze National Park, Poland, and found B1 as the most prevalent phylogroup.[30] In a review on potential impact of AMR in wildlife, environment, and human health, the phylogroups A and D were predominant among resistant *E. coli* strains against third-generation cephalosporins, B2 was predominant among susceptible strains to anti-microbials, and B1 was mostly able to persist in the environment; also, A phylogroup was considerably found in red foxes and birds of prey, and B1 phylogroup was predominant in yellow legged gulls, Iberian wolves, Iberian lynxes, and Passeriformes.[31] Phylogenetic analysis of *E. coli* isolates from wild animals in Thailand showed that phylogroup D was predominant in deer and elephants.[32] Distribution pattern of the phylogroups among *E. coli* strains isolated in various studies is dependent on the host species, host diet, body mass, and climate.[31]

This study revealed a high prevalence of phenotypic and genotypic resistant *E. coli* strains to β-lactams in wild hedgehogs living in urban regions of Kerman, Iran. The most important colicin genes were *E1*, *V*, *E2-E9*, and *Ia.Ib*. The strains were mostly classified into B1 and A phylogroups. Our findings suggest that hedgehogs could serve as potential reservoirs of anti-microbial-resistant *E. coli* for the environment. These results highlight the necessity of *E. coli* surveillance among wild animals.

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Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

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